Phenolphthalein

CAS #77-09-8
Swiss CD-1 mice, at 0.0, 0.1, 0.7, 3.0% in feed
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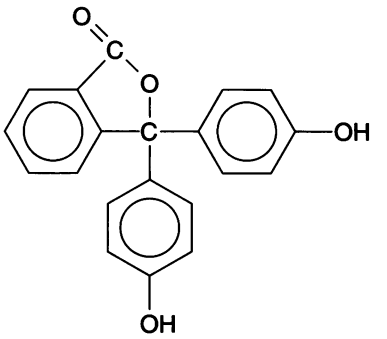

Phenolphthalein (P) has been widely used as a nonprescription laxative. It is not a laxative in mice. The potential reproductive toxicity of P was evaluated using Swiss CD-1 mice in the RACB protocol because of literature suggestions that P acts as a weak estrogen, and because data gathered at the end of a 90-day NTP study in B6C3F1 mice indicated adverse male effects. Concentrations of P in feed for this RACB study were 0.1, 0.7, and 3.0% weight per volume. Feed consumption dropped 8 and 4% for the middle and high dose groups, respectively, although body weights during Task 2 were unchanged at any concentration level. Based on body weight and feed consumption data, the estimated phenolphthalein intake for the low through high doses was approximately 0.15, 1.0, and 4.5 g/kg/day.

There was significant reproductive toxicity in F_0 mice. At the middle dose level, the proportion of pairs delivering a first through fifth litters was 100, *89, *84, *68, and *36% (* indicates significantly different from controls). The decline at the high dose was more severe; only 5% of pairs delivered a fifth litter. Overall, the mean number of litters per pair was reduced by 24 and 50% in the middle and high dose groups. Live pups per litter decreased by 58 to 59% in the middle and high dose groups; adjusted pup weight decreased by 10% only at the high dose. Cumulative days to litter increased by 12% for the medium dose and by 11% for the high dose animals for the second, third, and fourth litters.

The last litter from all dose groups was nursed by the dam until weaning, whereupon the F_1 mice were fed at the same concentration of P consumed by their parents. There was evidence of postnatal toxicity, as indicated by a 30 to 71% reduced survival at the high dose (n=3). All of the deaths occurred within the first 4 days postnatally. There was a nonsignificant increase in pup death in the middle dose group also, which may have contributed to the slight but significant approximately 18% increase in weight at the middle dose. Pup weight was reduced at the high dose by 9 to 40%, but this was statistically insignificant because only three litters were available.

The reproductive effects seen in Task 2 prompted the conduct of a crossover mating trial to determine the affected sex, using the control and middle dose mice. Although there was no difference among the groups with respect to the proportion of pairs mating or delivering pups, the group with 0.7% P-treated females delivered fewer than half the control number of live pups per litter, and took a day longer than the controls to do so. Neither the viability nor the pup weight adjusted for litter size was reduced in litters from treated females.

After the delivery of the crossover litters, the control and 0.7% P F_0 mice were killed and necropsied. In males, body weight was not changed, but absolute testis weight dropped by 36%, while epididymal weight was 8% less than control. Epididymal sperm density was reduced by 30%, while the proportion of abnormal sperm increased from 2.6 (control value) to 4% in the 0.7% P-exposed mice. In females, there was no treatment-related reduction in body or organ weights. Interestingly, there was also no change in the length or pattern of the estrous cycle. Histologically, the ovaries were unremarkable, while there was evidence of seminiferous tubule degeneration in 9 of 10 treated mice.

Because of excessive toxicity at the high dose, the reproductive effects on the second generation were evaluated only with the controls, low, and middle dose groups. At the time of mating, there were no treatment-related differences in body weights. In the middle dose group, only half the pairs that mated delivered any live young. Litter size was reduced by approximately 50%, although the viability of those pups and their body weight adjusted for litter size were not changed. No differences from control were seen at the low dose level.

Once the F_2 pups were evaluated and discarded, the F_1 adults in all dose groups were killed and necropsied. Changes were found only in the 0.7% P level. While male body weight was not affected, there were reductions in absolute testis weight (45%) and relative epididymis weight (15%). Sperm abnormalities increased from 2.2 (controls) to 4.9% (0.7% P); epididymal sperm density remained unchanged. In females, terminal body weight was reduced by 6%, though there were no changes in adjusted organ weights. Antemortem estrous cyclicity was unaffected by either level of P consumption. Histologic analysis of gonads revealed 7 of 10 testes with atrophy in the 0.7% P group and unremarkable ovaries.

In summary, phenolphthalein at these concentrations produced significant reproductive toxicity (fewer litters per pair, and fewer pups per litter) in the absence of changes in body or somatic organ weights. The second generation did not appear more affected than the first.

Summary: NTP Reproductive Assessment by Continuous Breeding Study.

NTIS#: **PB911787007**
Chemical: **Phenolphthalein**
CAS#: **77-09-8**
Mode of exposure: **Feed**
Species/strain: **Swiss CD-1 mice**

F_0 generation — Dose concentration →	0.1%	0.7%	3.0%
General toxicity	Male, female	Male, female	Male, female
Body weight	—, —	—, —	—, —
Kidney weight[a]	•	—, —	•
Liver weight[a]	•	—, —	•
Mortality	—, —	—, —	—, —
Feed consumption	—, —	↓, ↓	↓, ↓
Water consumption	•	•	•
Clinical signs	—, —	—, —	—, —
Reproductive toxicity			
x̄ litters/pair	—	↓	↓
# live pups/litter; pup wt./litter	—, —	↓, —	↓, ↓
Cumulative days to litter	—	↑	↑
Absolute testis, epididymis weight[a]	•	↓, ↓	•
Sex accessory gland weight[a] (prostate, seminal vesicle)	•	—, —	•
Epidid. sperm parameters (#, motility, morphology)	•	↓, —, ↑	•
Estrous cycle length	•	—, —	•

Determination of affected sex (crossover)	Male	Female	Both
Dose level	•	0.7%	•

F_1 generation — Dose concentration →	0.1%	0.7%	3.0%
General toxicity	Male, female	Male, female	Male, female
Pup growth to weaning	—, —	↑, —	—, —
Mortality	—, —	—, —	↑, ↑
Adult body weight	—, —	—, ↓	•
Kidney weight[a]	—, —	—, —	•
Liver weight[a]	—, —	—, —	•
Feed consumption	—, —	—, —	•
Water consumption	•	•	•
Clinical signs	—, —	—, —	•
Reproductive toxicity			
Fertility index	—	↓	•
# live pups/litter; pup wt./litter	—, —	↓, —	•
Absolute testis, epididymis weight[a]	—, —	↓, ↓	•
Sex accessory gland weight[a] (prostate, seminal vesicle)	—, —	—, —	•
Epidid. sperm parameters (#, motility, morphology)	—, —, —	—, —, ↑	•
Estrous cycle length	—	—	•

Summary information	
Affected sex?	Female
Study confounders:	None
NOAEL reproductive toxicity:	0.1%
NOAEL general toxicity:	0.1%
F_1 more sensitive than F_0?	No
Postnatal toxicity:	Yes

Legend: —, no change; •, no observation; ↑ or ↓, statistically significant change ($p<0.05$); —, —, no change in males or females. [a]Adjusted for body weight.